indicates the activation of wnt/β-catenin in both tissues. Wnt-1 induced signaling protein (WISP1), a protein downstream canonical wnt signaling, was highly expressed in the synovium as well, again indicating activation of this pathway. To determine whether canonical wnt expression in the synovium has the potency to cause cartilage damage, the canonical wnt wnt8a was overexpressed specifically in the synovium by intra-articular injection of an adenoviral vector. At day 1 and 3, no significant differences were observed in the cartilage from wnt8 overexpressing knee joints compared to joints transfected with control virus. Remarkably, at day 7, a strong induction of cartilage pathology was observed at the medial margin of the medial tibial plateau (Figure 1), a preferential site for the start of cartilage damage in our models. This shows that expression of canonical wnt in the synovium causes cartilage degeneration.

Due to their size, wnt proteins and WISP1 can reach the chondrocytes in the cartilage matrix and may alter the chondrocyte phenotype. Overexpression of wnt8, wnt16 and WISP1 in human chondrocytes led to the a significant increase within 14 days of Collagen type I, and a significant decrease of Collagen type II, suggesting degeneration of the chondrocyte phenotype.

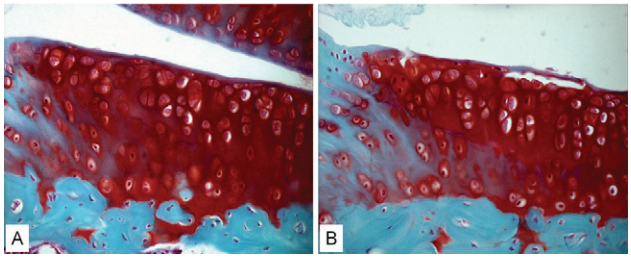

Figure 1. Focal OA-like cartilage degradation on the medial tibia after 7 days over-expression of wnt8a (B) in murine knee joints. Control virus did not induce pathology (A).

Conclusions: Canonical wnt expression and subsequent WISP1 is increased in the synovium during experimental OA. This synovial expression may lead to the degradation of cartilage, possible by dedifferentiation of the articular chondrocyte phenotype. This indicates synovial wnt expression as a potential target for OA therapy.

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CHONDROCYTE RESPONSIVNESS TO LEPTIN IS STRONGLY DEPENDENT ON THE BODY MASS INDEX OF PATIENTS WITH OSTEOARTHRITIS

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Purpose: Recent studies that have examined the relationship between body composition and cartilage structure indicate that metabolic factors associated with adiposity may contribute to the development and the progression of osteoarthritis (OA). Among adipose-derived proteins, namely adipokines, leptin has been identified as an important factor able to modulate chondrocyte functions. Although the physiological activity of leptin is to reduce food intake, obesity is characterized by an elevated systemic level of leptin which fails to decrease body weight, suggesting a leptin resistance in obese individuals. The current study has been therefore undertaken to determine whether hyperleptinemia found in the joint from obese OA patients induces also a defect in the action of leptin in chondrocyte.

Methods: Chondrocytes isolated from OA patients with various BMI ranging from 22 to 47 kg/m^2 were treated with 100 or 500 ng/ml of leptin. The expression of cartilage-specific components (aggrecan, type 2 collagen), as well as regulating factors (IGF-1, TGFβ, MMP-13, TIMP 2), was investigated by quantitative real-time PCR to evaluate chondrocyte responsivness to leptin.

Results: Addition of leptin to human OA chondrocytes up-regulates the expression of the genes encoding cartilage-specific components and regulating factors. However, the effect of the adipokine was shown to be strongly dependent on the concentration and the BMI of the patients. When both groups of OA patients were compared, chondrocytes obtained from normal or overweight patients (BMI30 kg/m^2). Based on this BMI-dependent leptin dose-response of chondrocytes, we demonstrated that the stimulating effect of leptin at 100 ng/ml was negatively related with the BMI of the patients while a positive association between chondrocyte responsivness and BMI was found at 500 ng/ml of leptin. Beside, the growth factor induced by leptin was also dependent on BMI. IGF-1 was up-regulated in chondrocytes collected from normal or overweight patients while mRNA level of TGFβ was increased in leptin-treated chondrocytes provided by obese patients. Moreover, the gene encoding MMP-13 was identified as a target of leptin for chondrocytes originated from obese patients only.

Conclusions: The current study showed that the BMI of OA patients changed the leptin dose-response of chondrocytes and indicated that chondrocytes from obese patients required elevated levels of leptin to be responsive. In addition, the BMI-dependent effect of leptin for the expression of growth factors and MMP-13 suggests that the adipokine may contribute to the fast progression of OA in obese individuals.

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ANALYSIS OF THE SECRETOME OF HUMAN ARTICULAR CHONDROCYTES INDUCED BY ACTIVATION OF TOLL-LIKE RECEPTOR 2

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Purpose: Toll-like receptors (TLR) are the major mediators of the innate immune response. In monocytes, macrophages and dendritic cells, the major effector cells, the primary response to TLR activation is the induction of inflammatory cytokines, chemokines and a varitety of matricellular proteins, which have been implicated in wound healing and tissue repair. In articular cartilage TLR activation, particularly that of TLR2, leads to expression of matrix metalloproteases and thus may contribute to cartilage erosion in osteoarthritis. However, the complete secretome of articular chondrocytes in response to TLR ligands has not been characterized. The purpose of this study was to analyse the proteins induced following activation of TLR2 and to determine if some of these components were present in human articular cartilage with early degenerative changes.

Methods: Human chondrocytes were exposed for 24 h to the TLR2 ligand peptidoglycan or TNF-α. Secreted proteins were analysed by mass spectrometry. The cytokine profile was analysed using a cytokine array. In addition, TNF-α levels in culture media were determined by Elisa assay. Induction of MMP3, MMP13, the Chi-lectins Chi3L1 and 2, and complement component C3 was further analysed by western blotting. Cartilage harvested from patients undergoing joint replacement (n=35, age range: 69 to 101 years old, 31 females, 4 males). following femoral neck fracture was extracted with guanidine. and e Extracts were analysed by western blotting for the presence of Chi3L1 and Chi3L2, and their levels were quantitated densitometrically and normalized to standards of purified protein included in each gel.

Results: Stimulation with the TLR2 ligand peptidoglycan led to increased production of the matrix metalloproteases MMP3 and